# Control of Virus Diseases of Stone Fruit Nursery Trees in New York

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# CONTROL OF VIRUS DISEASES OF STONE FRUIT NURSERY TREES IN NEW YORK[1]

R. M. GILMER, K. D. BRASE, and K. G. PARKER[2]

## Abstract

SYMPTOMS of the more important virus diseases found in New York occurring in nursery trees of the cultivated stone fruits are described in detail. These diseases affect sweet and sour cherry trees principally, but some of them also occur less commonly in peach and plum trees. Symptoms of certain diseases of unknown etiology and of certain genetic abnormalities with virosis-like symptoms are also described.

No single stone fruit index plant was found completely satisfactory under New York conditions for identifying all of the viruses commonly found in New York nursery trees. Three species, Montmorency sour cherry, Italian Prune, and Shiro plum, were necessary for analytical determination of virus content.

A comparison of indexing accuracy between small cucumber seedlings and Shirofugen flowering cherry plants showed good agreement between the two indexing methods in detecting infections with necrotic ring spot, sour cherry yellows, and green ring mottle viruses. Indexing with cucumber seedlings was found satisfactory for detecting latent infections with these viruses in sweet cherry, sour cherry, mazzard, mahaleb, and some plum varieties. The cucumber technique was not reliable for indexing myrobalan, Nanking cherry, damson plums, and certain ornamental species.

The principal disadvantage of the cucumber index method was lack of reliable differentiation among the various viruses. Some supplementary differentiation of some of these viruses was obtained with other cucurbit index plants such as squash and watermelon.

The history of the cherry certification program in New York nurseries is outlined. Since the adoption of certified budwood sources by commercial nurserymen, the incidence of sour cherry yellows in certified nursery trees has dropped to less than 1 per cent. The incidence of other common virus diseases, such as necrotic ring spot, has been reduced to less than 7 per cent.

Extensive indexing of commercial rootstocks indicated that these were the principal sources of virus contamination in New York nursery trees at the present time.

Procedures for the establishment and maintenance of a foundation planting for supplying virus-free budwood and details of the current New York certification regulations are supplied.

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[1]A joint contribution of the Departments of Plant Pathology and Pomology, Geneva, and the Department of Plant Pathology, Ithaca.

[2]The writers have drawn freely upon unpublished work of Dr. E. J. Klos, T. H. Barksdale, and Gustav Schmid in preparing this bulletin.

## Introduction

ONE OF THE most serious problems confronting the stone fruit industry, in both the nursery and orchard phases, is a group of diseases of virus origin. More than 50 virus diseases have been described on stone fruits, but only 10 or 12 of these are presently known to be of economic importance in New York. These diseases, alone, cause an annual loss in orchard and nursery trees and in fruit production amounting to at least $1,000,000. This estimate would undoubtedly be increased if the economic injury caused by several of the less well-known virus diseases were adequately assessed.

Many of the important stone fruit viruses are commonly carried in nursery trees, but a few are not. X-disease, peach yellows, and little peach viruses are carried only rarely in nursery trees produced in New York. Since these three diseases either kill orchard and nursery trees outright or cause stunted or atypical growth, affected trees are avoided by nurserymen in selecting propagating materials. Nursery sites are generally located in areas removed from wild and cultivated stone fruit trees, so that the introduction of X-disease virus from wild chokecherries and of peach yellows and little peach viruses from wild or cultivated peaches and plums is avoided. Because these diseases are of primary concern to the orchard grower rather than to the nurseryman, they are not described in this bulletin.

Several important stone fruit viruses are, however, easily carried in nursery trees. Among these viruses are those causing necrotic ring spot, sour cherry yellows, green ring mottle, prune dwarf, line pattern, and vein-clearing rosette. These viruses decrease bud-take in the nursery and reduce the growth of infected nursery trees. Some of them impair fruit quality in the orchard—others do not. Diseased trees produce small fruit crops and are often more subject to cold injury than are healthy trees.

These virus diseases are especially common in sweet and sour cherry trees and some of them are occasionally found in peaches and plums. The causal viruses spread from tree to tree in the orchard and are often introduced into new plantings directly with nursery trees.

Infections in nursery trees almost always result from methods used in propagation. The stone fruit viruses invade most or all of the tissues of infected trees, so they are readily carried in buds, grafts, cuttings, and other propagating materials. Some of them are transmitted directly through seeds to young plants used as rootstocks. When such infected rootstocks are propagated with healthy scions, the virus rapidly pervades the entire tree.

## Need for Control of Virus Diseases in Nurseries

Because fruit trees are perennial plants with a normal life expectancy of 15 to 20 years or more, the cumulative effects of early virus infection are particularly serious. Once infected, a tree remains infected for the remainder of its life. At the present time there is no practicable method of eliminating a virus from an infected tree, so that if virus diseases are to be controlled in the orchard, they must first be adequately controlled in the nursery. Control in the nursery is possible only by preventing infection. All parts of the nursery tree, rootstock and scion, must be derived from virus-free sources.

Although a healthy nursery tree may be subsequently infected after establishment in the orchard, setting a new orchard planting with healthy trees affords an important advantage. Delayed infection permits the tree to approach or reach maturity and to develop a good fruiting spur system before the disease can affect it. The rate of spread of virus diseases can be slowed considerably by planting healthy trees in large solid blocks. Even under conditions most conducive to virus spread, such as interplanting in old orchards where many diseased trees exist, if healthy trees are planted, infection with sour cherry yellows virus is often delayed by several years. During the life of an orchard established with virus-free nursery trees, the total fruit production may be conservatively estimated to range between 25 and 50 per cent greater than in an orchard planted with virus-infected trees. Such an advantage is one that the commercial fruit grower cannot afford to ignore. Because of the demonstrated superior performance of virus-free trees in New York, the demand for them is already substantial and will continue to increase.

The most obvious ill effects of some virus diseases do not become apparent until the trees begin to bear fruit. In New York, sour cherry yellows decreases yield capabilities 40 to 50 per cent if trees become infected while very young. Green ring mottle causes such a serious impairment in fruit quality as well as in fruit yield that fruit of infected trees is often not harvested. Both of these viruses may be carried in nursery trees and it is particularly important that they not be introduced directly into new orchard plantings.

In addition to the losses resulting from setting orchards with infected nursery trees, virus diseases also cause serious direct losses in the nursery planting itself. If diseased buds are used for propagation, the percentage of bud-take is often reduced (Table 1) with occasional complete failures. Additional losses result even when the diseased buds unite with the understocks and grow, since the growth of the resultant budlings is likely to be subnormal (Table 1 and Figs. 1 and 2). Reduc-

TABLE 1.—BUD-TAKE AND GROWTH OF MONTMORENCY CHERRY NURSERY TREES PROPAGATED FROM VIRUS-FREE BUDS AND FROM BUDS INFECTED WITH NECROTIC RING SPOT VIRUS (NRSV), GENEVA, N. Y., 1956.

| Bud source | Number of trees budded | Percentage bud-take | Percentage 1-year trees in grades* | | | |
|---|---|---|---|---|---|---|
| | | | Extra | No. 1 | No. 2 | Unsalable |
| Healthy....... | 674 | 84.0 | 17 | 42 | 34 | 7 |
| NRSV........ | 130 | 72.3 | 13 | 34 | 44 | 9 |

*Grades classed as follows:
Extra = Trunk diameter (caliper) 11/16–12/16 inch
No. 1 = Trunk diameter (caliper) 9/16–10/16 inch
No. 2 = Trunk diameter (caliper) 7/16–8/16 inch
Unsalable = Trunk diameter (caliper) below 7/16 inch

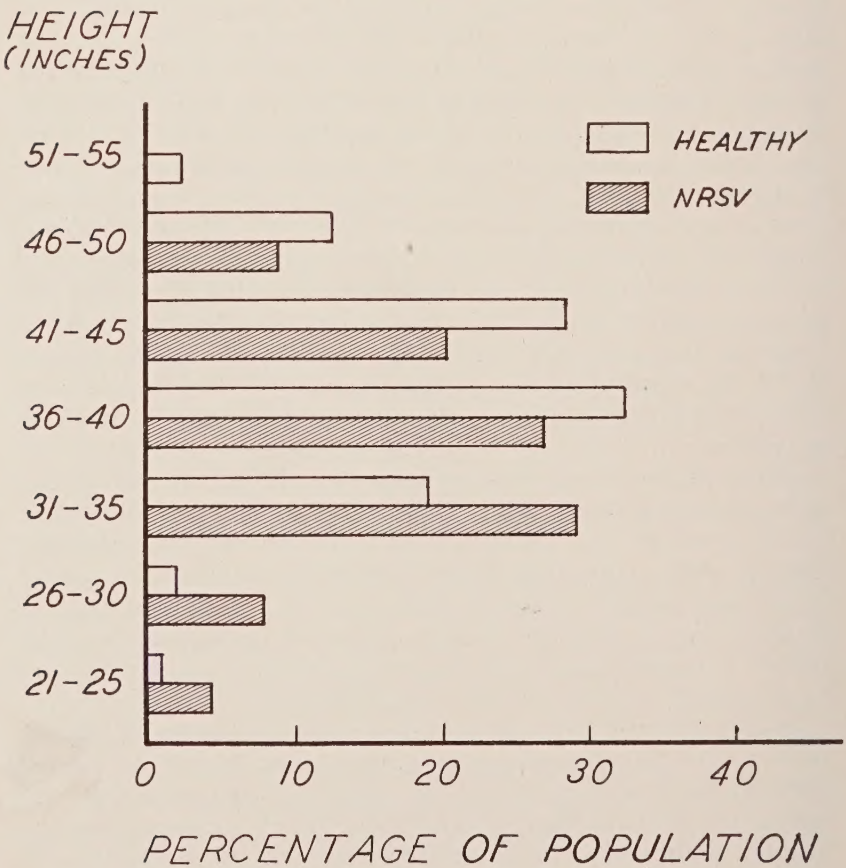


Fig. 1.—Height of one-year Montmorency cherry nursery trees propagated with virus-free and with necrotic ring spot infected (NRSV) budwood.

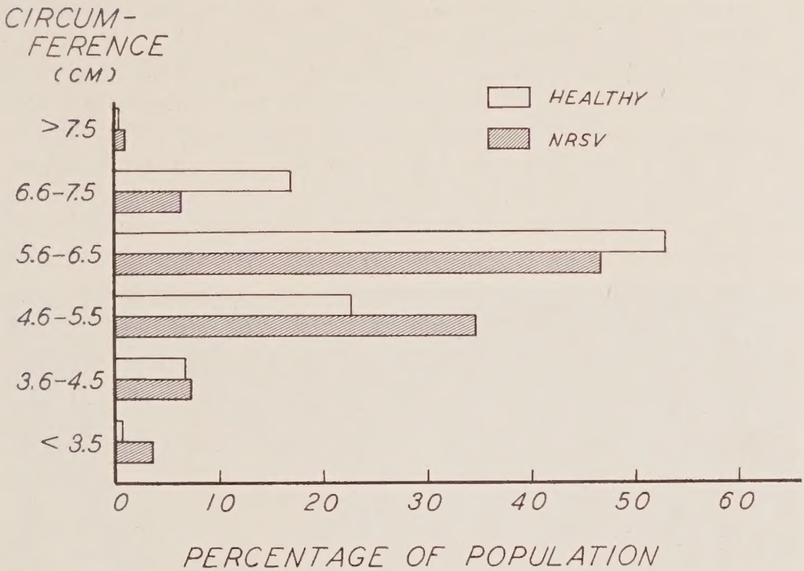


Fig. 2.—Trunk circumference of one-year Montmorency cherry nursery trees propagated with virus-free and with necrotic ring spot infected (NRSV) budwood.

tion in percentage bud-take and in growth of infected budlings has been found dependent, in large degree, upon the particular virus or virus strain present (20).[3]

Indirect losses from virus diseases are also incurred by nurserymen because of the reluctance of fruit growers to plant trees with possible virus infections. Certain fruit varieties are shunned because of low or erratic fruit production which, in many cases, results from virus infections. Certain species of stone fruit nursery trees produced in New York are excluded from some states by quarantines against virus diseases imposed by governmental agencies of those states.

## Important Virus Diseases of Stone Fruit Nursery Trees

### Sour Cherry Yellows

Sour cherry yellows is the most damaging of the virus diseases of sweet and sour cherry trees in New York. Whether this disease is caused by a single virus or by a complex of two or more viruses has not yet been resolved. Symptoms and severity of injury of the disease in both nursery and orchard trees are variable, indicating the occurrence of

[3]Refers to Literature Cited, page 52.

numerous virus strains. Some of these differences may be attributable to superimposed infections with other viruses.

Sour cherry yellows virus can infect practically all species and varieties of the stone fruits but is most prevalent in sweet and sour cherry varieties. Symptoms of the disease are not diagnostic in sweet cherries, but all of the commonly grown sour cherry varieties (Morello, Montmorency, and Early Richmond) show the characteristic yellow-leaf symptom when infected. Moore and Keitt (23) have shown that the development of the yellow-leaf symptom in infected sour cherry trees

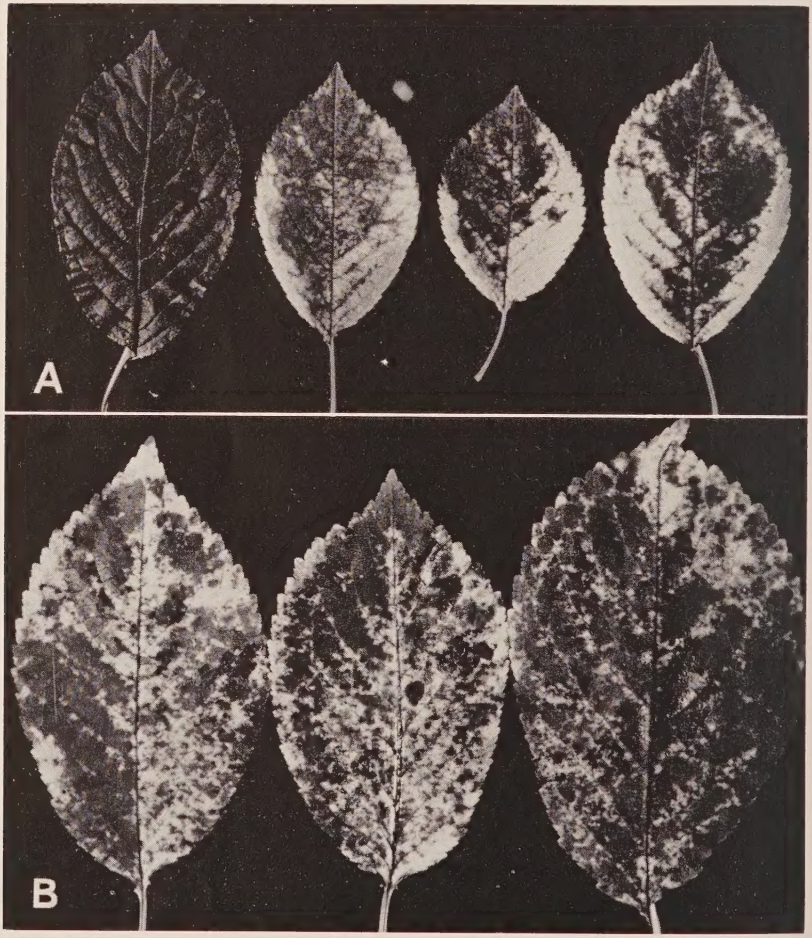


Fig. 3.—Symptoms of sour cherry yellows: A, early season (mid-June) symptoms in Montmorency foliage; B, late season (mid-August) symptoms in Montmorency foliage. The mottle is cream to white in color rather than lemon yellow as in the early season symptoms.

occurs only if the trees are exposed to low temperatures (61° F). In fruiting trees, the most critical time for this temperature effect occurs during the 30-day period immediately after bloom (21), and a similar time-growth relationship occurs in nursery trees.

In young sour cherry nursery trees, diagnostic symptoms of sour cherry yellows are transient and of short duration. One or more leaves become irregularly mottled with green and yellow (Fig. 3) during the second or third week of June under conditions normally present in western New York. The mottled leaves drop early and, after the loss of such leaves, the infected trees show no other symptoms except reduced growth. Leaf mottle and abscission may be completely absent if high temperatures occur early in the growing season.

Sour cherry yellows virus is spread in the nursery either through the use of diseased buds or by propagating with healthy buds on diseased rootstocks. The virus or virus complex is seed-transmissible (4, 5). Although an insect vector undoubtedly is the primary agent of spread of this virus in orchard plantings, practically no spread by this means occurs in the nursery.

## Necrotic Ring Spot

Necrotic ring spot is the most common virus disease of orchard and nursery cherry trees. Although the causal virus may occur alone, it is frequently found associated with other viruses. It has been postulated by several workers (3, 9, 22) that necrotic ring spot virus is an essential component of the sour cherry yellows complex, but direct evidence to this effect is lacking. Numerous strains of necrotic ring spot virus exist, as evidenced by variation in symptoms and severity of effect on host plants.

Like the sour cherry yellows virus, necrotic ring spot virus can infect practically all species and varieties of stone fruits. The virus is most commonly found in sweet and sour cherry varieties. Peaches and plums are relatively free of the virus in the nursery.

Leaves of infected sour cherry trees in the nursery show watersoaked or chlorotic rings, arcs, and lines, or they may show water-mark patterns. Affected areas of the leaves are often killed outright and the dead areas frequently drop out (Fig. 4). Affected leaves are often smaller than normal in size and the margins are often wavy or ruffled. The above syndrome is commonly referred to as the acute or "shock" phase of the disease. In severe infections, dieback of the growing points of the terminals may occur. Terminal elongation is commonly sharply reduced, the distance between successive leaf axils is shortened, and the entire tree becomes temporarily rosetted.

Fig. 4.—Symptoms of necrotic ring spot on Montmorency foliage. *Lower row,* acute or "shock" symptoms with considerable necrosis of foliage; *upper row,* almost symptomless leaves produced by an infected tree the year after acute foliage symptoms appeared.

Duration of the acute phase of necrotic ring spot is usually brief and is frequently followed by apparent complete recovery. The new leaves produced are symptomless, or almost so, and quasi-normal growth is resumed. Montmorency cherry trees that have recovered from the acute phase often do not show further evident symptoms.

Symptoms in sweet cherry varieties are, in general, much like those described in sour cherries (Fig. 5). Following the acute stage of the disease, leaf symptoms persist in certain sweet cherry varieties but not in others. If symptoms are persistent, they are usually not as severe as the initial symptoms.

Necrotic ring spot virus is transmitted in the nursery in the same ways that sour cherry yellows virus is spread. Again, although the virus spreads rapidly in orchard plantings through the probable agency of some insect vector, vector transmission does not appear to be an important means of spread of necrotic ring spot virus in the nursery.

## Green Ring Mottle

Green ring mottle may be readily identified in fruiting Montmorency cherries by the presence of chlorotic constrictions along the

Fig. 5.—Symptoms of necrotic ring spot on Black Tartarian sweet cherry foliage. The holes in the lower leaves are caused by loss of necrotic tissue.

leaf veins and by the occurrence of yellow leaves prominently marked with deep green blotches or rings (Fig. 6). The yellow-leaf symptom is usually evident about 2 to 3 weeks after symptoms of sour cherry yellows appear, i.e., normally during the first 2 weeks of July in western New York.

Parker and Klos (25) have associated discoloration and necrotic pitting of Montmorency fruits with infection by green ring mottle virus (Fig. 7). Infection by this virus, although not resulting in readily identifiable foliage symptoms in Morello, has been associated with the presence of small, late-maturing fruits in that variety (25).

Green ring mottle is not easily identified in nursery plantings, since foliage symptoms on young sour cherry trees are likely to be obscure or absent. Although several species of stone fruits have been experimentally infected with the virus, it has as yet been found naturally occurring in New York orchards only in sour cherries.

The virus is spread in the nursery principally through the use of infected propagating materials. Whether it is seed transmitted is not known, although it may well be. Green ring mottle virus spreads relatively slowly in orchard plantings.

Fig. 6.—Symptoms of green ring mottle on Montmorency foliage. *Right,* characteristic yellowing with prominent dark green blotches or rings; *center,* a leaf showing the constricting chlorosis symptom; *left,* a healthy leaf.

Fig. 7.—Fruits of Montmorency cherry showing discoloration and necrotic pitting associated with green ring mottle. The fruits at extreme right are from a healthy tree.

## Prune Dwarf

The prune dwarf virus can be diagnosed definitely only in certain plum and prune varieties, such as Italian Prune (Fellenberg), Lombard, Tragedy, and Albion. Although prune dwarf virus causes a serious disease in prunes, natural infections in prunes are quite rare in New York. The virus is, however, very commonly found in sweet and sour cherry varieties and in damson plums. None of these latter hosts show diagnostic symptoms of infection. Prune dwarf virus is very common in nursery and orchard sour cherry trees with sour cherry yellows.

In an appropriate indicator host, such as Italian Prune or Albion plum, infection with prune dwarf virus results in the production of characteristic strap-shaped leaves (Fig. 8). Affected leaves are thickened, somewhat rugose, and tend to be brittle. The venation is often more prominent than in normal leaves of the same variety. Symptom expression in infected trees is governed by temperature, and symptoms are completely masked in foliage produced when temperatures are 75° F and above. In nursery plums, discrete symptoms are often confined to the first five to six leaves on a vigorously growing terminal for this reason.

The prune dwarf virus moves quite slowly in large plum and prune trees, and diagnostic symptoms of the disease may be confined to a single branch for several years. Normal shoots occasionally appear in trees that had previously expressed symptoms on all branches.

Prune dwarf virus is transmitted in the nursery largely through the use of diseased propagating wood. Whether the virus is seed-transmitted is not known.

## Line Pattern

Like prune dwarf, line pattern can be diagnosed with certainty only in certain plum varieties. The characteristic symptoms are best observed in certain Japanese (*Prunus salicina* Lindl.) plum varieties, such as Shiro and Becky Smith, although some European (*P. domestica* L.) varieties and myrobalan (*P. cerasifera* L.) seedlings often show identifiable symptoms after infection.

Infection with line pattern virus most commonly occurs in sweet and sour cherry varieties, in which diagnostic symptoms of the disease are not produced. The virus can often be isolated from sour cherry trees that have shown symptoms of sour cherry yellows.

In diagnostic hosts such as Shiro plum, the foliage of infected trees is mottled prominently with green and bright yellow during the early spring (Fig. 9). Later in the growing season, with the advent of warmer temperatures, the new foliage does not show symptoms, although

Fig. 8.—Foliage of Italian Prune with symptoms of prune dwarf. Note the misshapen leaves, the prominence of the veins, and general rugosity.

Fig. 9.—Line pattern symptoms in Shiro plum foliage the second year after experimental inoculation from an infected Montmorency cherry.

these persist in the older foliage formed under cooler conditions. The virus patterns in the foliage are often of the water-mark or oak-leaf type, but they may consist only of irregular blotches or merely of prominent vein-clearing. Line pattern virus moves rather slowly in infected plum trees and recognizable symptoms may not appear until 2 years after inoculation.

Line pattern virus is spread in the nursery by the use of diseased propagating wood. The possibility of seed transmission has not been fully investigated.

## Diseases of Unknown but Probable Virus Etiology

Because of the inherent slowness of the development of symptoms of some diseases of stone fruits, several diseases have been observed whose etiology is uncertain. Among such diseases is a midsummer drop of peach fruit which is very probably of virus origin, but the disease is of little apparent economic importance and will not be described. Although diseases of this type are of potential significance, their increase and dissemination in nursery trees should be prevented by the current nursery improvement program. Two other disorders of stone fruits are, however, already of considerable economic importance, although their etiology has not been fully determined.

### Vein-clearing Rosette of Cherry

This disease has been present in New York for at least 15 years or longer, having originally been described by Hildebrand (15) as rosette or vein-clearing of cherry. The field occurrence in sour cherry and grower experience with the disease indicate that, although the vein-clearing rosette has been present for many years, spread in most orchards is comparatively slow.

Affected Montmorency sour cherry trees grow in an abnormally upright manner with few lateral shoots or fruiting spurs. Terminal growth is shortened and somewhat rosetted. The leaves are smaller than normal, dull in appearance, and slightly misshapen, with prominently serrated margins (Fig. 10). The leaf veins tend to be lighter in color than the surrounding tissues. Early in the growing season, foliage color is distinctly lighter green than in normal trees, and in severely affected trees the entire tree shows a yellowish cast. Blossoms are restricted almost entirely to the terminals and open 3 to 5 days later than on normal trees. A very low percentage of blossoms set fruit. Fruit pedicels are shorter than normal, and the fruit may be deformed on the suture side. Quality of fruit has not been thoroughly studied, but there is no obvious impairment of flavor or decrease in sugar content. Affected trees bear such light fruit crops that they fail to repay the cost of picking.

Many affected trees, particularly in certain old orchards, develop leaf yellowing followed by defoliation—symptoms almost identical with those of sour cherry yellows. Leaf yellowing and subsequent defoliation may, however, continue to develop throughout the entire growing season. Whether this yellowing and defoliation are actually symptoms of vein-clearing rosette or whether such trees are merely additionally infected with sour cherry yellows virus is not known.

Inoculation tests indicate that vein-clearing rosette is caused by a transmissible virus, with the early stages of infection characterized by intensification of the leaf symptoms previously described. Inoculations from sweet cherry trees with leaf symptoms similar to those described for sour cherries produce what is apparently the acute form of the disease in sour cherry trees. Symptoms in sweet cherries have some of the characters of Pfeffinger disease, a widespread and serious virus disease of the sweet cherry in Europe.

Vein-clearing rosette may be spread in the nursery by the use of buds from affected trees in propagation. No information on seed transmission of the virus has been obtained. If cherry nursery trees are produced only with certified scionwood, it seems quite feasible to exclude this disease from new orchard plantings and to eliminate it gradually from the entire State.

Fig. 10.—Symptoms of vein-clearing rosette (Pfeffinger disease?) in Montmorency sour cherry. *Right,* a terminal from an infected tree. Note the prominent leaf serrations and vein-clearing (inset) as compared to the healthy terminal at left.

**Constriction Disease of Stanley Prune**

The Stanley prune, introduced in 1926, has been widely accepted as a valuable commercial variety in New York and other states. Shortly after the end of World War II, however, many New York growers reported that numerous young trees in orchard plantings were showing unthrifty growth and subsequent death.

Certain trees 2 to 3 years after establishment in orchards become unthrifty, although prior to that time many of them have made vigorous growth. The foliage of affected trees first becomes pale yellowish green in color and the trees fail to make new terminal growth. In severe cases, the trees wilt during the growing season. Affected trees may die almost immediately or may decline progressively for one or two years before death occurs.

In such unthrifty or dying trees, the main shank of the myrobalan rootstock is considerably smaller than the scion trunk immediately above the point of union (Fig. 11). Such constrictions usually occur just below the ground line, so that they are not visible in a cursory inspection. Suckering of the myrobalan rootstock is common on affected trees.

Preliminary indications of poor rootstock growth and the initiation of constriction may be evident in the young nursery tree (Fig. 12), but, more frequently, evident constriction does not occur until after the young tree has been established in the orchard. In the early stages of the disorder the union of the scion and rootstock is smooth and well formed, but in the later stages, longitudinal sections through the union show that the phloem tissues of the Stanley scion and the myrobalan rootstock are not well united. Death of the bark tissue ultimately occurs, appearing first in the bark of the rootstock just below the union with the scion.

Constrictions similar in appearance to those occurring in some Stanley trees propagated on myrobalan invariably appear when Stanley is propagated on American plum (*Prunus americana* Marsh.) rootstocks. The percentage of Stanley trees propagated on myrobalan that develop constriction, however, varies with different lots of myrobalan rootstocks. The development of constriction in Stanley trees propagated on myrobalan stocks has been associated by Brase and Parker (1) with the occurrence in myrobalan stocks of a leaf condition which they termed chlorotic fleck (Fig. 13). This condition was initially described by Hildebrand (14) as asteroid spot or chlorotic spot of myrobalan. He was unable to transmit the causal agent of the condition.

Recent observations indicate that a chlorotic spotting of myrobalan

Fig. 11.—Constriction of the myrobalan rootstock of a Stanley prune tree, showing marked decline four years after setting in the orchard. The myrobalan stock is suckering heavily as a result of the decline of the affected Stanley top.

Fig. 12.—A two-year Stanley Prune tree on myrobalan with the bark removed to show the initiation of constriction.

results from feeding injury by an eriophyid mite, *Vasates fockeui* (Nal. & Trt.).[4] According to Kiefer (17), this mite is morphologically indistinguishable from the peach silver leaf mite, *V. cornuta* (Banks). The latter mite has been shown to cause yellow flecking of peach foliage (26). The true cause or causes of the chlorotic flecking of myrobalan have not yet been determined with certainty.

Hildebrand (14) reported that myrobalan seedlings with chlorotic fleck made reduced growth and that Fellenberg (Italian Prune) budlings propagated on such seedlings also grew subnormally. Brase and Parker (1) found, in addition to Fellenberg, that Stanley and Abundance budlings also made less growth on stocks with severe chlorotic fleck than on stocks with very little chlorotic fleck evident. Only Stanley trees, however, showed any evidence of the initiation of constriction at the bud union, and the amount of constriction was much more severe in trees propagated on stocks with severe chlorotic fleck than in trees propagated on stocks with very light chlorotic fleck (1).

The exact causes of constriction disease of Stanley trees are still unknown. There is some preliminary evidence that certain Stanley selections are more prone to constriction disease than other selections of this variety. The fact

[4]Identification made by H. H. Kiefer, California Department of Agriculture.

Fig. 13.—Chlorotic fleck of myrobalan foliage.

that orchards of Stanley trees propagated on myrobalan which were established in the period 1930 to 1940 are unaffected by the disorder indicates that it does not result solely from scion-stock incompatability. The subnormal growth of Fellenberg and Abundance buddings propagated on myrobalan rootstocks with chlorotic fleck indicates some abnormality in such rootstocks. There is, however, no direct evidence of virus causation at the present time.

In recent years, a concerted effort to select improved myrobalan seed trees has been made by experiment station workers in the Pacific Coastal region. The writers are testing certain of these selections for

freedom from the factor or factors that cause constriction disease in Stanley prune. Certain plum rootstocks other than myrobalan are also being tested.

## Diseases with Virosis-like Symptoms Probably not Caused by Transmissible Factors

A group of stone fruit disorders with virosis-like symptoms have not been demonstrated to be transmissible. Some, or all, of these disorders may be heritable abnormalities, and in the one disorder that has been intensively studied, there is strong evidence that it is of genetic origin.

### Sweet Cherry Crinkle

Leaves of affected sweet cherry trees are distinctly atypical in shape, with irregular and often deeply indented margins (Fig. 14). Chlorotic patches or streaks often extend from the bases of such indentations into the leaf lamina, paralleling the lateral veins. Within the chlorotic areas, the leaf lamina appears to have failed to grow laterally, giving the impression of lateral compression of the tissue or of crowding of the lateral veins. Fruits borne by affected trees are pointed in shape, with the suture mcre prominent than usual. Fruits often color prematurely but actually tend to ripen later than fruits on normal trees.

Fig. 14.—Symptoms of sweet cherry crinkle in foliage of Black Tartarian. *Left,* a normal leaf of the variety; *right,* two leaves from a nursery tree affected with crinkle.

The cause of this disorder has not been transmitted from an affected tree to a normal one by the accepted techniques of inoculation, but sweet cherry crinkle can be readily perpetuated when buds of affected trees are used to propagate new nursery trees. The appearance of prominent foliage symptoms in young nursery trees may be delayed for several years. Seedlings from both affected and nonaffected trees may show symptoms of crinkle, and strong evidence has been presented that this disorder is actually a genetic abnormality (16).

Crinkle has been found in New York only in Black Tartarian and Bing. Neither is an important commercial variety in the State.

Under the present nursery improvement program in New York, scionwood is selected from foundation trees maintained under continuous observation. Since crinkle is apparently not a transmissible disorder, careful scionwood selection should eliminate it from nursery trees grown in New York.

### Crinkle Leaf of Prune

The leaves of affected prune trees closely resemble those found in sweet cherry trees affected with sweet cherry crinkle. The margins are irregular, and chlorotic areas are often found associated with deep indentations of the leaf margins. Often only a single branch or portion of a branch on an otherwise normal tree expresses symptoms. Fruit set is very sparse on affected trees or branches. Italian Prune is the only important commercial variety grown in New York known to be affected by crinkle leaf.

Limited inoculations indicate that prune crinkle leaf is not caused by a transmissible agent. The disorder can be readily eliminated by scionwood selection.

### Leaf-casting Mottle of Prune

The symptoms of this disease resemble in some respects those of a prune disorder called leaf spot of prune in the western United States. Of the plum and prune varieties commonly grown in New York, leaf-casting mottle is most common and most severe on Italian Prune (Fellenberg), but the disorder also occurs on other varieties such as Albion, Tragedy, Grand Duke, and Hungarian prune. Leaf-casting mottle has not been found on Stanley prune.

Chlorotic spots develop in midsummer on the leaves of affected trees. Shortly after the initiation of these spots, they become brownish yellow and translucent, with indefinite margins. Still later, the leaf lamina between the spots becomes yellow, leaving a green halo surrounding each individual spot. At this stage, the center of the spots

often becomes brown and necrotic, and the entire leaf soon drops (Fig. 15). The fruit set of affected trees is lighter than in normal trees, and much of the fruit that is set drops prematurely during the summer. On severely affected trees very little fruit remains at harvest (2).

Symptoms tend to increase in severity with high temperatures. In 1955, however, under conditions of abnormally high temperatures and

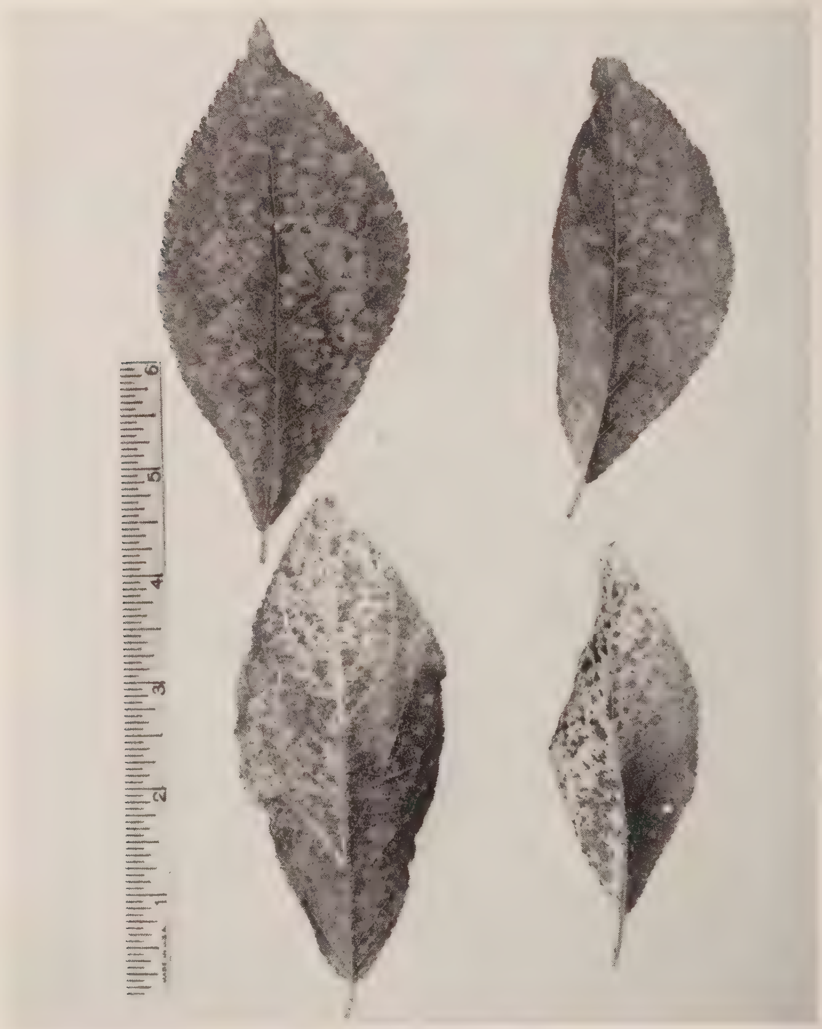

Fig. 15.—Leaf casting mottle of Italian Prune. The upper leaves show the translucent spotting; the lower leaves show loss of chlorophyll except in areas near the sites of the translucent spotting.

severe drouth, heavy leaf and fruit drop occurred without the development of pronounced mottle symptoms on the foliage.

Numerous attempts to transmit the causal agent of the disease by budding or grafting with material obtained from severely affected trees have failed. Symptoms invariably develop on the scions, but none appear on the tree in which the grafts were placed. Symptoms very similar to those of leaf-casting mottle are often found in plum seedlings, particularly in seedlings derived from crosses in which Italian Prune, Tragedy, or Grand Duke are parents. It thus seems probable that leaf-casting mottle, like sweet cherry crinkle, is due to a genetic abnormality.

Mottle-free selections of Italian Prune are being distributed to New York nurserymen for propagations.

### Warty Suture of Peach

Warty suture of peach, originally described in Washington and Oregon, has been encountered in only one orchard in New York. Only a few trees showed symptoms of the disease. Fruits of affected trees produce a wart-like proliferation of tissue along the suture (Fig. 16). The proliferated tissue becomes reddish in color as the fruits mature. The flavor of affected fruits is poor, and the fruits themselves are unsightly.

Trees affected by warty suture can be propagated by taking scion-wood from an affected tree, but no causal agent has been transmitted to a healthy tree by budding or grafting with material obtained from affected trees. This disease likewise appears to be of genetic origin.

A somewhat similar disorder was discovered in the same peach orchard where warty suture was observed. The fruits of affected trees showed large depressions irregularly distributed over the surface. The affected fruits failed to ripen normally and softened without developing normal flavor. This condition was also perpetuated by propagation, but no causal agent was transmitted to healthy trees by accepted inoculation techniques.

## Methods of Detecting Latent Virus Infections

### General Considerations

Several of the stone fruit viruses may be present in infected trees without readily evident indications of their presence. Certain viruses that cause prominent symptoms in one stone fruit variety cause no evident symptoms in a different variety or species. Infections of this latter type are commonly termed "masked infections" and the infecting virus is termed "masked" or "latent".

Fig. 16.—Warty suture of Hale Haven peach. The wart-like overgrowths are confined to the suture.

Still others of the stone fruit viruses incite only transient symptoms in certain host plants. The presence and severity of such symptoms is often governed by the occurrence of critical temperatures at some appropriate period of growth of the host plant. In the absence of such critical temperatures, determinative symptoms of infection fail to occur.

At the time when new nursery trees are propagated in midsummer or late summer, symptoms of infection by several of the more important stone fruit viruses are completely absent. Thus, the presence or absence

of harmful viruses cannot be determined by mere visual examination of scionwood or of understocks.

The presence of such masked or latent viruses can be detected by introducing them into a healthy host plant that will indicate their presence by reacting to infection with well-defined and characteristic symptoms. Such host plants are termed "index" hosts, and the entire process of determining the presence or absence of masked viruses by their use is termed "indexing".

In one type of indexing, buds are obtained from the tree about which virus information is desired. Two or more of these buds are inserted into each of several appropriate indexing host plants. After a suitable incubation period, the length of which is largely determined by the individual virus, characteristic symptoms of disease appear in the index hosts. If the source tree from which the buds were obtained is virus-free, the index host plants remain healthy.

In New York, such index hosts are usually budded in the early autumn, and symptoms usually become evident at or shortly after budbreak the following spring. With certain slow-moving viruses, such as those of line pattern and prune dwarf, characteristic symptoms of infection may not appear until two or more growing seasons have elapsed after inoculation. This extended indexing period can often be shortened by a double-budding technique. An appropriate rootstock (myrobalan or peach) is budded about 6 inches above the ground line with a bud of the index host in late July. After the index bud has united, usually a matter of 10 to 14 days, buds from the source tree about which virus information is required are inserted just below the index bud. The entire rootstock is permitted to grow for the remainder of the season. Following the winter dormant period, the top of the rootstock is removed just above the point where the index bud has united. The index bud is thus forced into very vigorous growth. If virus was present in the source tree buds, symptoms appear almost immediately on the index shoot (Fig. 17). Prune dwarf and line pattern viruses can almost invariably be detected during the first season following inoculation by this method. Albion or Italian Prune are varieties chosen as indexing hosts for prune dwarf virus, while Shiro plum is used to index for line pattern virus.

An ideal index host is one that can be grown rapidly and reliably from seeds, that attains a usable size within a short period of time, and that responds to virus infection rapidly and with diagnostic symptoms. Among the numerous plants that have been tested in New York are peach *(Prunus persica* Batsch) seedlings from several sources, Montmorency sour cherry (*P. cerasus* L.), Shirofugen flowering cherry *(P.*


Fig. 17.—The double-budding technique used for prune dwarf indexing in the field. A, healthy Italian Prune scion on a peach rootstock. The pencil rests where the healthy bud was inserted. B, Italian Prune scion with symptoms of prune dwarf growing on a peach rootstock. The pencil rests where the diseased bud was inserted. Both photographs taken in June following double-budding the previous September.

*serrulata* Lindl.), Kwanzan flowering cherry *(P. serrulata),* mazzard seedlings (*P. avium* L.), Albion and Italian Prune (*P. domestica* L.), Becky Smith and Shiro plums (*P. salicina* Lindl.), Nanking cherry (*P. tomentosa* Thunb.), and cucumber (*Cucumis sativus* L.) and squash (*Cucurbita pepo* L.). Each of these index plants possesses some special advantage, but no single plant has proved entirely satisfactory as an index plant when used alone. Commonly, two or more of these species are used in combination.

### Stone Fruit Virus Indexing With Stone Fruit Indicator Plants

**Peach as an indexing plant.**—Peach seedlings have been used very extensively in New York as index plants for necrotic ring spot and sour cherry yellows viruses since they were initially advocated by Hildebrand (13). Under usual field conditions, peach seedlings react to inoculation with necrotic ring spot virus with delayed foliation, dieback of the terminals, and ring spotting and necrosis of the foliage. If sour cherry yellows is present in the inoculum, rosetting and inten-

sification of green foliage color occur in addition to symptoms of necrotic ring spot (Figs. 18, 19). The distinction between severe necrotic ring spot reactions and mild sour cherry yellows reactions can be made only with considerable difficulty, since symptoms of the two diseases frequently intergrade. Other viruses, such as line pattern virus and prune dwarf virus, cannot be determinatively diagnosed on peach seedlings. Peach seedlings are often not satisfactory indexing hosts under greenhouse conditions, where symptoms of virus infection are usually not pronounced.

For the development of most pronounced symptoms under field conditions, peach seedlings should be inoculated in early to mid-September after growth has almost ceased. Symptoms are most pronounced shortly after budbreak the following spring. Trees infected with necrotic ring spot virus tend to recover from the acute phase of the disease and resume quasi-normal growth within a period of 2 to 3 weeks, but those infected with sour cherry yellows usually remain perceptibly rosetted for some time.

Indexing inoculations may also be made in the very early spring just as growth starts. Symptoms appear within 3 to 4 weeks, but are usually not as pronounced as when inoculations are made in the fall. Midsummer inoculations may also be made if the inoculated trees are pruned back to within two to four nodes above the inoculating buds. Symptoms develop on the new shoots within a period of 4 or 5 weeks.

Of the several peach varieties whose seedlings have been tested, Muir seedlings appear to be slightly superior indexing plants. Seedlings of Lovell peach are routinely used in New York, but they are more susceptible to powdery mildew than are Muir seedlings and do not react as strongly to necrotic ring spot virus infection as do Muir seedlings. Athough seedlings of Rio-Oso-Gem peach are very satisfactory indexing plants, the seeds of this variety usually germinate poorly.

Among the principal advantages of peach seedlings as indexing plants are the facts that peach seeds are easily obtained from commercial sources and that the seedlings grow vigorously, reaching usable size within 3 months after the seeds germinate. Peach seedlings make satisfactory rootstocks for both Italian Prune or Shiro plum, the varieties used in the double-budding technique to detect prune dwarf and line pattern viruses.

Germination of commercially obtained peach seeds, however, is often poor or erratic. Peach seedlings are very subject to injury by peach tree borers [*Sannoidea exitiosa* (Say) and *Synanthedon pictipes* (G. & R.)], and peach aphids [*Myzus persicae* (Sulz.)]. Seedlings of many varieties are susceptible to powdery mildew, which may obscure virus symptoms

Fig. 18.—Indexing on Lovell peach seedlings in the field. Seedling at left received a bud from a tree infected with sour cherry yellows and prune dwarf viruses. Seedling at right received a bud from a healthy tree. Both plants were cut back to a single 4-inch shoot in the early spring while still dormant.

Fig. 19.—Indexing on Lovell peach seedlings in the field. Plants in foreground were budded with buds carrying prune dwarf and sour cherry yellows viruses. Plants in the background were budded with healthy buds. Note that the latter plants are knee-high.

if indexing is done in midsummer. Cochran (7) has shown that peach ring spot virus, certainly very similar to if not identical with necrotic ring spot virus of cherries (24), is transmitted through peach seeds to seedlings.

**Nanking cherry as an indexing plant.**—Fink (8) reported that indexing with Nanking cherry seedlings under Iowa conditions detected strains of necrotic ring spot virus not detected in parallel indexing on Elberta peach budlings. Nanking cherry has been tested in New York and was found to be of value as an indexing host. The response to necrotic ring spot virus is much like that occurring in peach seedlings —delayed foliation, terminal dieback, and necrosis or ring-spotting of the foliage. The presence of sour cherry yellows virus in the inoculum is often indicated by the persistence of rosetting, but the intergradation of symptoms between necrotic ring spot and sour cherry yellows makes distinction between the two diseases very difficult. The reactions to such viruses as those of prune dwarf, green ring mottle, and line pattern are not diagnostic. Nanking cherry seedlings are quite useful indexing plants in the greenhouse, where symptoms of virus infections are much more evident than on peach seedlings. Like peach seedlings, however, Nanking cherry seedlings may fail to react with evident symptoms to all strains of the necrotic ring spot virus (11).

Nanking cherry seedlings are very hardy, and are not attacked by any important insect or fungus pests in the field. Seeds and seedlings are commercially available. The major disadvantage of this species as an indexing plant is that seedlings often require two growing seasons in the field before attaining usable size for indexing purposes.

It is highly probable that seed transmission of some of the stone fruit viruses occurs in this species, but the point has not been determined.

**Mazzard *(P. avium)* seedlings as indexing plants.**—Mazzard seedlings from virus-free seed trees provide good index plants for necrotic ring spot virus. Symptoms of infection by the other common stone fruit viruses are not diagnostic. Inoculations may be made in early September with symptoms developing the following spring, or they may be made in the early spring as growth starts, in which case symptoms develop within a period of 4 to 5 weeks. Midsummer inoculations also provide good indexes if the seedlings are pruned back to two to four nodes above the inoculating buds. Symptoms develop on the new shoots within 3 to 5 weeks.

Although commercial sources of mazzard seeds are available, seeds obtained from such sources carry relatively high percentages of the seed-transmissible viruses (6). Mazzard seedlings require two seasons of growth before reaching usable size and are somewhat difficult to

transplant under field conditions. They are very susceptible to infestations by the black cherry aphid [*Myzus cerasi* (F.)] and to infections of cherry leafspot and powdery mildew during the summer.

**Montmorency sour cherry as an indexing plant.**—Indexing on plants of Montmorency sour cherry, a clone of *P. cerasus*, detects practically all strains of necrotic ring spot virus. This variety is also a diagnostic host for green ring mottle and sour cherry yellows viruses. Symptoms of sour cherry yellows often do not develop until the second season after inoculation, and the appearance of prominent symptoms of green ring mottle may be delayed even longer. Leaves of young trees infected with green ring mottle virus, however, usually show constricting chlorosis along the veins by the second year after inoculation. Symptoms develop reliably only if necrotic ring spot virus is present with green ring mottle virus in the index tree.

The symptoms of necrotic ring spot virus infection are very similar to those already described for peach and Nanking cherry seedlings. The most pronounced symptoms appear at budbreak following inoculation the previous autumn. Inoculations made with necrotic ring spot virus during midsummer (late July to early August) when the Montmorency index tree has almost, but not quite, completed growth, often fail to result in discernible symptoms even though the virus invades the tree systematically.

Although Montmorency budlings are widely used as index plants, they are relatively expensive. Since the variety cannot be grown true from seeds, all indexing plants must be multiplied vegetatively, either by budding or grafting. Understocks and scionwood, unless known to be virus-free, must themselves be indexed prior to use. Commercially available trees are often infected. Montmorency budlings in the field are subject to infestations of the black cherry aphid and to attack by the fungi causing leafspot and powdery mildew.

**Shirofugen flowering cherry as an indexing plant.**—The variety Shirofugen, a clone of *P. serrulata*, is a very sensitive index host in detecting necrotic ring spot virus. Nearly all strains of this virus are lethal to Shirofugen index plants. Necrosis of the tissue accompanied by a copious gumming occurs at the sites where infected buds are inserted (Figs. 20, 21). Ultimately, that portion of the terminal beyond the point of bud insertion is killed.

A similar response occurs when the inoculating buds contain other viruses, such as those of sour cherry yellows, prune dwarf, and green ring mottle, indicating that the inoculating buds additionally contain necrotic ring spot virus or that the Shirofugen index plants do not provide a differential response to infection with these different viruses.


Fig. 20.—Indexing on Shirofugen flowering cherry. (A) Union and growth of healthy sweet cherry buds on Shirofugen, approximately 1 year after budding. (B) The characteristic reaction to necrotic ring spot virus. Gummosis and tissue-killing are progressing down the Shirofugen shoot. (Photograph B taken 44 days after budding).

Although indexing on Shirofugen does not detect all strains of necrotic ring spot virus (11, 18), this variety is one of the most efficient index hosts.

The necrotic response of Shirofugen plants to infection with several stone fruit viruses is initially localized and the invading viruses move very slowly in the host tissues. Thus, as many as 25 to 30 indexings can be accomplished simultaneously on the various branches of a single 3-year-old tree.

The major disadvantage of Shirofugen is that it must be multiplied by vegetative propagation. The variety can be grown readily from softwood cuttings, or it may be propagated on mazzard seedlings by budding or grafting. Since Shirofugen buds fail to unite or to grow on rootstocks infected with necrotic ring spot virus, there is little danger

Fig. 21.—Indexing on 4-year-old Shirofugen trees in the field. The dead and dying (arrows) branches resulted from inoculations made the previous summer.

of producing a prospective index plant infected before use. Shirofugen trees in the field are quite free of important insect and fungus pests.

**Kwanzan flowering cherry as an indexing plant.**—Although Kwanzan, also a clone of *P. serrulata,* is not generally as sensitive an indexing plant as Shirofugen for detecting strains of necrotic ring spot virus, Milbrath (18) has shown that it may reveal the presence of strains not detected by Shirofugen. Necrotic ring spot virus rapidly invades Kwanzan index plants systematically, in contrast to the initially localized infections that occur in Shirofugen.

Kwanzan is an efficient index plant for detecting the presence of green ring mottle virus, provided that necrotic ring spot virus is already present in the index plant or accompanies green ring mottle virus. Symptoms of green ring mottle usually appear the season following that of inoculation (Fig. 22). They consist of twisting of the leaf petioles and of veinal necrosis. Callus tissue later appears at the sites of veinal necrosis, and the formation of callus usually results in a twisting of the entire leaf lamina. Following the initial watersoaking and necrosis of the leaf veins, the affected vein segments dry out and remain evident for the remainder of the season.

Like Shirofugen, Kwanzan plants must be multiplied by vegetative propagation. Kwanzan may be readily grown from softwood cuttings

Fig. 22.—Symptoms of green ring mottle in Kwanzan flowering cherry.

or the variety may be propagated on mazzard understocks by budding or grafting. There are no important fungus or insect pests.

**Albion and Italian Prune as index plants.**—Albion and Italian Prune, varieties of European plum *(P. domestica),* are specialized index hosts used to determine the presence of prune dwarf virus. They are also fairly useful for detecting necrotic ring spot virus, which causes ring-spotting and necrosis of the foliage similar to symptoms caused in sour cherry. Index tests are usually made by the double-budding technique which results in the production of very pronounced prune dwarf symptoms on the foliage of the young budlings early in the spring.

Both of these varieties can be propagated either on peach or myrobalan seedlings. They are subject to attacks of various aphids and, to some degree, by plum leafspot (*Coccomyces prunophorae* Hig.), although this disease is usually not serious in New York.

**Shiro plum as an indexing plant.**—Shiro plum, purportedly a complex cross of *P. salicina* × *P. simoni* × *P. cerasifera* × *P. munsoniana* but whose tree and fruit characters are typical of *P. salicina*, is a special index host used to detect the presence of line pattern virus. Tests are usually made by the double-budding technique which results in the production of pronounced symptoms of line pattern on the foliage

of the young budlings early in the spring. Unlike the other stone fruit index hosts, Shiro budlings often fail to show symptoms of infection with necrotic ring spot virus.

This index host can be propagated on either peach or myrobalan seedlings. It is occasionally attacked by aphids, but is otherwise free of important pests.

**Other special indexing plants.**—A number of species and varieties of *Prunus* are in use on the Pacific Coast to detect the presence of certain viruses. Among the more important index hosts are Bing sweet cherry (for K & S virus, rusty mottle virus, and mottle leaf virus) and Lambert sweet cherry (for Lambert mottle virus). None of these viruses has yet been found in New York, with the possible exception of K & S virus, so that these special index hosts have not been used extensively. However, their inclusion in the standard host range is indicated for indexing foundation sources of propagating materials.

**Disadvantages of stone fruit index plants.**—All of the enumerated index hosts are themselves stone fruits. They are subject to several disadvantages.

1. They must be derived from virus-free sources or must be indexed prior to use as index plants.
2. Many of them must be propagated by vegetative means, an expensive and time-consuming process. Where seedlings can be used, the seeds must be derived from virus-free source trees. Seeds require extended periods of stratification before they germinate, and the germination percentage is often low.
3. Symptoms of virus infection frequently develop very slowly, the time required for symptoms to appear after inoculation ranging from 3 weeks to several months. Symptoms of certain diseases may not appear for 2 years or more.
4. Occasional aberrant virus strains are not detected.

## Stone Fruit Virus Indexing With Cucurbits

Plants of several genera of the Cucurbitaceae are excellent index hosts for certain of the stone fruit viruses. Cucurbits are readily grown from seeds and the seedlings reach an appropriate size for indexing use within a short period of time, usually within 7 to 10 days after the seeds are planted. Since none of the cucurbits have been found naturally infected in the field with any of the viruses of stone fruits, erroneous stone fruit index readings because of prior infection of the index plants do not occur.

Many of the most common stone fruit viruses are mechanically transmissible to cucurbits, but a few either are not infective or do not cause evident symptoms. However, stone fruit trees infected with viruses such as those of necrotic ring spot, sour cherry yellows, prune dwarf,

and green ring mottle can be readily indexed on certain of the cucurbits.

**Cucumber seedlings as indexing plants.**—Of the several cucurbit species tested in New York, seedlings of cucumber (*Cucumis sativus* L.) are the most useful for general indexing. Certain varieties of squash (*Cucurbita pepo* L.) and watermelon (*Citrullus vulgaris* Schrad.) are useful as special indexing plants.

Cucumber seedlings of several varieties (National Pickling, Chicago Pickling, Ohio 17, Ohio MR 31, Straight 8) are susceptible to infection by certain of the stone fruit viruses. The latter may be transmitted by mechanical inoculation of cucumber cotyledons with juice inocula obtained from immature foliage of the trees being indexed. Cucumber seedlings are most susceptible to infection when inoculated while the first true leaf is still in bud. With age, they progressively become more resistant to infection, and if inoculation is delayed until after the first true leaf is fully expanded seedlings seldom become infected systemically.

Cucumber indexing was reported the most efficient of indexing methods tested with 126 stone fruit virus isolates carried in peach seedlings (11). The reliability of the cucumber method when used for direct indexing from sour cherry trees was tested in February, 1957. A total of 103 Montmorency cherry trees, some healthy and others diseased, were indexed by forcing dormant buds in the greenhouse, triturating the young foliage, and inoculating young cucumber seedlings with the resultant juice extracts. Parallel indexing of the same group of trees was performed by budding young Nanking cherry seedlings. The same group of 103 Montmorency trees had been previously indexed in the summer of 1956 on Shirofugen plants.[5]

The results of the three indexing methods are given in Table 2. Although some isolates were detected by one indexing method and not by either of the others, in general, the agreement among the three methods was good. As reported in indexing from peach (11), the cucumber method was the most efficient in detecting virus infection in Montmorency cherries.

Cucumber seedlings are not diagnostic hosts for differentiating the individual stone fruit viruses that infect them. Symptoms in infected cucumber seedlings were much the same for a group of 126 stone fruit virus isolates containing several viruses in various combinations (12). Differences in symptom severity and in symptom type do occur, but such differences have not been conclusively related to infection by particular viruses (Fig. 23).

[5]Data by courtesy of T. H. Barksdale and Gustav Schmid.

TABLE 2.—THREE INDEXING METHODS FOR DETECTING VIRUS INFECTIONS IN MONTMORENCY CHERRY TREES, 1956–1957.

| NUMBER OF TREES GIVING INDICATED REACTIONS | SHIROFUGEN CHERRY* | NANKING CHERRY† | CUCUMBER† |
|---|---|---|---|
| 62 | –‡ | – | – |
| 2 | – | – | + |
| 5§ | – | + | + |
| 27 | + | + | + |
| 1 | + | + | – |
| 3 | + | – | – |
| 3 | + | – | + |
| Total positive reactions | 34 | 33 | 37 |
| Percentage efficiency in virus detection‖ | 83 | 81 | 90 |

*Indexing accomplished in summer, 1956.
†Indexing from dormant trees, winter 1956–57.
‡The sign (–) indicates no virus detected; (+) indicates virus detected.
§Some of the discrepancy between the Shirofugen index and those of Nanking cherry and cucumber may be due to infections that may have occurred subsequent to the Shirofugen index.
‖Percentages based on summation of number (41) of diseased trees detected by all methods combined.

Fig. 23.—Indexing on cucumber seedlings in the greenhouse. Plants at center and right were inoculated with foliage extracts of two different diseased trees; those at left from a healthy tree.

Not all species of stone fruits can be indexed on cucumber seedlings. Among the species that can be reliably indexed in this manner are mahaleb, peach, sour cherry (varieties Montmorency, Early Richmond, and Morello) and sweet cherry (most varieties). Italian Prune can also be indexed fairly reliably on cucumber seedlings. Other species of stone fruits, among them myrobalan, Nanking cherry, and certain

ornamental species, cannot be indexed on cucumber seedlings. The reasons for the failure to transmit necrotic ring spot or other viruses from the foliage of infected plants of these species are obscure. The most plausible explanation of the failure is that the juice extracts of these species contain inhibiting substances that prevent infection of cucumber seedlings.

Similar inhibitors occur in the foliage of most sour and sweet cherry varieties, but the inhibition of virus infection of cucumber seedlings can be avoided or minimized if juice inocula are obtained only from very young leaf tissues.

**Squash seedlings as indexing plants.**—Milbrath (19) and Gilmer (12) have reported that certain varieties of squash seedlings were differential host plants for some stone fruit viruses. Milbrath found that seedlings of the variety Buttercup developed bright golden yellow leaf patterns following inoculations from cucumber seedlings infected with seven isolates containing sour cherry yellows virus obtained from peach foliage. Other stone fruit virus isolates similarly transferred from peach to cucumber and from cucumber to squash either developed local lesions only, a mild chlorotic mottle, a severe necrosis of the growing point, or no symptoms.

Gilmer (12) reported the occurrence of a bright golden yellow vein-banding and vein-clearing in seedlings of Cocozelle squash inoculated directly from foliage of peach trees infected with various stone fruit viruses (Fig. 24). Although the three virus isolates that produced this syndrome in Cocozelle seedlings all contained sour cherry yellows virus, these same isolates additionally contained line pattern virus. Since other stone fruit virus isolates containing sour cherry yellows virus but not line pattern virus did not produce this syndrome in Cocozelle seedlings, he concluded that line pattern virus probably caused the vein-banding syndrome.

Additional data obtained recently have definitely proved that the line pattern virus is not the cause of the vein-banding syndrome in Cocozelle squash seedlings, and it is possible that the virus that produces this syndrome in Cocozelle squash is actually sour cherry yellows virus. The vein-banding syndrome may be masked or obscured, however, if other viruses are contained in the inoculum in addition to that of sour cherry yellows.

**Watermelon seedlings as indexing plants.**—Young watermelon seedlings (variety Honey Cream) are differential host plants useful for the detection of the presence of an as-yet unidentified stone fruit virus. The majority of virus isolates obtained from sour cherry foliage do not produce evident symptoms of infection in watermelon seedlings, but

Fig. 24.—The veinbanding syndrome on Cocozelle seedlings 13 days after inoculation. Veinbanding occurs only in the second true leaf of each plant, the first true leaves being symptomless.

inoculation with certain isolates results in the production of small, pin-point necrotic lesions on the cotyledons. These lesions tend to increase in size, and systemic invasion of the seedling growing point follows, usually resulting in collapse and death of the entire plant.

The identity of the virus that causes this syndrome in watermelon seedlings has not yet been determined. On the basis of presently available data, it appears highly unlikely that this symptom results from infection with either necrotic ring spot virus or sour cherry yellows virus.

## Steps in the Indexing Procedure Practiced in New York

In order to lengthen the period when indexing can be performed and to conserve the more expensive stone fruit index plants, preliminary indexing is usually carried out with cucumber seedlings in the greenhouse during the period from late February through late May or early June.

Budsticks may be removed from trees at any time while the tree is dormant following completion of the necessary rest period. The sticks preferably should be 8 to 10 inches long, and should be selected from the most vigorous terminals. The bases of the budsticks are inserted in

tap water, and approximately an eighth of an inch is trimmed off the bases every 3 to 5 days until the young foliage appears. Two or three small leaves are then removed, triturated in 1 to 2 ml of distilled water, and the resulting juice extract is rubbed gently on the cotyledons of cucumber seedlings. The cotyledons should be dusted lightly with 400-mesh carborundum before inoculation to facilitate establishment of infection.

At least four seedlings are customarily inoculated for each tree under test. If the foliage extracts contained necrotic ring spot or sour cherry yellows virus, symptoms of infection appear within 5 to 8 days after inoculation in plants held at 70 to 80° F. If no symptoms appear in any of the inoculated plants within 12 days, the tree is presumed virus-free.

Confirmatory tests are then made by indexing the presumptive healthy trees on a stone fruit index host. Shirofugen index plants are most commonly used, since they are the most sensitive of the stone fruit index plants. This index host can be budded at any time between June and August in New York and readings obtained the same season. Alternative to the use of Shirofugen, peach, Nanking cherry, or Montmorency cherry may be used for indexing. These index plants are customarily inoculated in early to mid-September and the indexing plants left in the field until the following spring when readings are made.

The number of budsticks required to index a tree properly is dependent upon the size of the tree. With nursery trees 3 years of age or less, a single budstick will provide a reliable index. With larger trees, a single budstick is taken from each quarter of the tree, since a newly infected tree of large size may not be completely invaded throughout within a single season. All indexings should be performed at least in duplicate when stone fruit index hosts are used or replicated on four to eight cucumber plants if the latter are used for indexing. The inclusion of appropriate diseased and healthy check plants in each indexing series affords an indication of when symptoms should appear.

Since the principal aim of the nursery improvement program in New York is the provision of virus-free propagating materials for commercial usage, these indexing tests are satisfactory in that they indicate whether viruses commonly found in New York are present or absent. The tests do not necessarily differentiate between individual viruses that may be present. Trees not eliminated from further consideration in these indexing tests are then further indexed on such diagnostic plants as Montmorency, Kwanzan, Italian Prune, and Shiro plum. Bing, Lambert, and Napoleon are varieties recently added to the diagnostic host range to detect the viruses of rusty mottle, Lambert mottle,

small bitter cherry, and K & S disease, which, as yet, are not known to occur in New York.

## Effect of Certification on Quality of Stone Fruit Nursery Trees

When the nursery improvement program was initiated in New York in 1939, its principal objective was the elimination of sour cherry yellows in sour cherry nursery trees. At that time, little was known about the virus diseases of stone fruits or the incidence of virus infection in nursery trees.

The incidence of sour cherry yellows in sour cherry nursery stock during this period was observed to be very high (15). Diseased trees were frequently found in groups of from four to seven consecutive plants in the nursery row, indicating that such a group had been propagated from a single diseased budstick. Hildebrand (15) found that the incidence of sour cherry yellows was highest in the variety Chase, but other sour cherry varieties also showed high incidences of infection (Table 3). Hildebrand's estimates were based on visual observation, so that he did not estimate the incidence of latent infections with other viruses.

Table 3.—The Incidence of Sour Cherry Yellows in Various Sour Cherry Varieties in New York Nurseries, 1939.*

| Variety | Number of trees inspected | Percentage of trees visibly infected with sour cherry yellows |
|---|---|---|
| Chase | 2,995 | 95 |
| Early Richmond† | 910 | 3 |
| Early Richmond‡ | 1,102 | 90 |
| English Morello‡ | 496 | 51 |

*Table compiled from data of E. M. Hildebrand (15).
†Propagating budwood obtained from nursery trees.
‡Propagating budwood obtained from orchard trees.

On the basis of field observations, Hildebrand recommended that propagating wood be selected from nursery trees rather than from bearing trees in the orchard. Since the incidence of virus infection was lower in nursery than in orchard trees, this recommendation was generally adopted by nurserymen and resulted in an appreciable decrease in number of infected trees produced in the nursery. The variety Chase was completely abandoned (15).

Additional control of sour cherry yellows was attempted by a stringent roguing program carried out by horticultural inspectors of the New York State Department of Agriculture and Markets. The roguing

program during the period 1943 to 1951 resulted in the annual removal of about 20 per cent of Montmorency nursery trees because of visible infections of sour cherry yellows. Removals of trees of the varieties English Morello and Early Richmond were somewhat less, ranging from 15 to 25 per cent in the case of English Morello and about 5 per cent in the case of Early Richmond.[6] These percentages remained relatively constant over the 8-year period.

It had become evident by 1949 that the selection of propagating materials in the nursery supplemented by roguing of the obviously diseased trees had failed to provide an acceptable degree of control of sour cherry yellows in the nursery. In a series of indexing tests in 1949, 55 of 216 Montmorency nursery trees selected at random in four commercial nurseries were found to be diseased by indexing on peach seedlings—a virus incidence of 25.5 per cent. Similar indexing of approximately 50 trees of each of four sweet cherry varieties showed a virus incidence ranging from 30 to 89 per cent, dependent upon the variety. Further data had meanwhile become available on the adverse effects of green ring mottle and necrotic ring spot on orchard trees. These data indicated the necessity of initiating control of these diseases in nursery trees where symptoms of infection were likely to be obscure. It was also highly desirable to expand the virus control program to sweet cherry and plum varieties in which the obscurity or absence of virus symptoms in nursery trees rendered roguing very difficult.

An extensive indexing program that included the important commercial varieties of sweet cherries, sour cherries, and plums was undertaken. Trees were selected in the nursery row for conformity to varietal type and for excellent vigor. Following indexing, virus-free selections were established in a foundation planting located at Geneva.

Substantial amounts of budwood were first released to the cooperating nurseries in 1950. The cooperating nurseries established special primary nursery blocks as future sources of propagating wood with budwood obtained from Geneva. These blocks, of necessity propagated on nonindexed rootstocks, were intensively rogued to eliminate infections that originated in the rootstocks. Scionwood obtained from the budlings in the primary blocks was then used for commercial propagations. More than 197,000 buds from the Geneva foundation planting have been released to commercial nurserymen for the establishment of primary blocks during the period 1950 to 1956.

By 1953 to 1954, the first seasons in which large numbers of trees derived from Geneva foundation budwood were produced, the observed

[6]Data on roguing were supplied by Louis Rhind, W. E. Ozard, H. P. Breitfeld, and Howard Swanson, horticultural inspectors of the New York State Department of Agriculture and Markets.

field incidence of sour cherry yellows in Montmorency nursery trees fell from approximately 20 per cent observed in 1951 to less than 1 per cent. The actual total virus incidence when tested by actual indexing was somewhat higher than the observed figure (Table 4). The

TABLE 4.—VIRUS INCIDENCE DETECTED BY SHIROFUGEN INDEX PLANTS IN MONTMORENCY NURSERY TREES FROM COMMERCIAL NEW YORK NURSERIES, 1956.*

| Nursery | Number of trees indexed | Percentage diseased |
|---|---|---|
| Certified | | |
| A | 100 | 5 |
| B | 50 | 2 |
| C | 100 | 7 |
| Average | | 5.2 |
| Non-certified | | |
| D | 50 | 6 |
| E | 49 | 22 |
| F | 49 | 4 |
| G | 50 | 12 |
| H | 50 | 4 |
| Average | | 9.7 |

*All trees were propagated from bud sources that originated in the Geneva foundation plantings. Trees in the noncertified class were not eligible for certification because of insufficient nursery isolation or because budwood sources were more than 2 years removed from the initial propagations from foundation budwood sources.

method of indexing used, however, detected latent infections of other viruses in addition to those of sour cherry yellows virus. The percentage of infected trees agreed fairly well with the known virus incidence in commercial rootstocks (Table 5). Further improvement in the control of virus diseases of the stone fruit nursery stocks must await the advent of virus-free commercial rootstocks in quantity.

TABLE 5.—INCIDENCE OF VIRUS INFECTIONS IN COMMERCIAL MAHALEB ROOTSTOCKS AS REVEALED BY CUCUMBER INDEXING, 1957.

| Lot number | Seed source | Number of seedlings indexed | Percentage infected |
|---|---|---|---|
| A | New York | 100 | 7 |
| B | France* | 200 | 18 |
| C | Pacific Coast | 100 | 9 |
| D | Pacific Coast | 100 | 11 |
| E | Pacific Coast | 100 | 6 |
| Average | | | 11.5 |

*Seeds labeled French imported; seedlings grown in New York.

## Selection and Maintenance of Virus-Free Stone Fruit Varieties in Geneva Foundation Planting

Young trees of the various commercial stone fruit varieties are selected from commercial nursery plantings or Experiment Station plantings on the basis of conformity to type and vigorous growth. These trees are then indexed on cucumber seedlings and on the stone fruit index host range to detect possible latent virus infections. Trees that index negative are then provisionally established in the foundation planting, which is located in an isolated area removed as far as possible from other stone fruit trees.

Following the provisional establishment of the young tree in the foundation planting, it is carefully observed throughout the growing season for foliage or growth abnormalities. In addition to visual inspections, the tree is re-indexed annually on one or more types of index plants during the entire period that the tree is maintained as a budwood source. If no foliage or growth abnormalities appear, and if two successive index tests indicate absence of viruses, budwood for propagation is released. Any tree that shows foliage or growth abnormalities or indexes positive for virus content is immediately removed from the planting. During the period from the establishment of the foundation planting in 1949 through June, 1957, only 5 trees of 140 have been eliminated because of virus infection after establishment in the isolation planting.

Unless eliminated for foliage abnormalities or virus infection, all trees entering the isolation planting are maintained until they reach fruiting age. They are then allowed to fruit and are examined to determine whether they are typical of the variety under whose label they are carried.

Experience gained in the maintenance of the Geneva foundation planting has emphasized the necessity of having large numbers of young trees available as budwood sources. Particularly in the case of sour cherry varieties and to a lesser extent with other species of stone fruits, trees more than 7 years of age become unsatisfactory sources of budwood for propagating use. Although the extremely severe pruning associated with the supply of large amounts of budwood stimulates vigorous new vegetative growth, trees more than 5 years of age differentiate increasing numbers of flower buds on the current season's growth. In contrast to the other stone fruits, the flower buds of cherries fail to develop vegetative shoots when propagated, and such propagation is a complete failure in producing new trees. It has thus been found necessary to repropagate new cherry trees for the foundation

planting at least every 5 years in order to maintain the original varietal selections in condition to supply large amounts of acceptable budwood.

Repropagation of sweet and sour cherry varieties for the foundation planting has been found most successful when mazzard *(P. avium)* seedlings are used as rootstocks. Mazzard rootstocks result in increased vigor of budlings and these are slower to reach fruiting age than budlings propagated on mahaleb seedlings. Mazzard seedlings are also more tolerant of heavy, poorly drained soils than are mahaleb seedlings.

In addition to supplying adequate soil fertilization, an effective program for the control of insects and diseases is essential to maintain the trees in vigorous condition.

## Virus-Free Varieties Currently Available

Major emphasis has been devoted to obtaining sour and sweet cherry varieties free of viruses and genetic abnormalities, since these two stone fruits are most severely affected by such troubles in New York. Virus-free budwood of six selected Montmorency clones, including the Musselman, McClain, and Gilbert selections, is available for propagating purposes. Other virus-free sour cherry varieties available are Early Richmond, English Morello, and Meteor.

Virus-free budwood of the sweet cherry varieties Black Tartarian, Bing, Emperor Francis, Napoleon (Royal Anne), Schmidt's Bigarreau, and Windsor is obtainable. The varieties Lambert and Hedelfingen have been screened and young trees readied for establishment as future budwood sources. Other varieties are currently being indexed.

A virus-free clone of Italian Prune that is also free of leaf-casting mottle and prune crinkle leaf and a virus-free clone of Lombard plum are also available. Stanley prune is being screened to obtain clones that are free of constriction disease. Selected seed trees of virus-free mahaleb, mazzard, myrobalan, and Mirabelle also are maintained.

## Certification Regulations

Any New York nurseryman can produce stone fruit nursery trees that are eligible for certification by the New York State Department of Agriculture and Markets as having been produced from virus-free budwood sources. He should comply with the following simple regulations:

1. Buds from the indexed source trees of the Geneva virus-free foundation planting may be obtained for propagating a *primary* nursery block. The *primary* block should be isolated by a distance of at least 100 feet from all other stone fruit nursery trees produced from non-indexed scionwood and by at least 400 feet from mature stone fruit trees or wild species of stone fruits. (Stone fruits are

defined as including all species of the genus *Prunus*, i.e., sweet cherries, sour cherries, Duke cherries, Japanese plums, European plums, *P. americana* plums and hybrids, apricots, peaches, and such ornamental species as *P. besseyi, P. glandulosa, P. japonica, P. maritima, P. padus, P. pissardi, P. tomentosa,* and *P. triloba.* Wild species include chokecherry, pin cherry, black cherry, wild mazzard, wild plum, and wild mahaleb.)

2. Propagating wood may be taken for a period not to exceed 2 years from the *primary* block and used to propagate trees in a *secondary* block which should be isolated by at least 100 feet from stone fruit nursery trees propagated from non-indexed scionwood and by at least 400 feet from mature stone fruit trees or wild species of stone fruits. Trees from both *primary* and *secondary* blocks are eligible for certification as having been produced from virus-free propagating wood. Propagating wood from *secondary* blocks cannot be used to propagate additional certified trees.
3. Both primary and secondary blocks are subject to inspection by a Horticultural Inspector of the New York State Department of Agriculture and Markets who will remove and destroy any diseased trees.
4. Any block with a total virus incidence exceeding 7 per cent *or* a sour cherry yellows incidence of more than 1 per cent will not be eligible for certification.

## Virus Incidence in Commercial Cherry Rootstocks

The present certification program merely assures that the scionwood used to initiate the production of certified trees is virus-free. The propagation of such scionwood on virus-infected rootstocks will obviously result in the production of diseased trees even though regulations dealing with budwood sources and isolation are strictly complied with.

The incidence of virus infection in various lots of commercial mahaleb rootstocks obtained from New York nurseries is given in Table 5. There was little difference in the virus incidence in stocks grown from seeds harvested from wild mahaleb trees in New York and those received from the Pacific Coast, an area from which the majority of mahaleb understocks used in New York are obtained. The incidence in stocks grown from seeds obtained from Europe was, however, twice that found in stocks grown from American seed sources (10).

The incidence in mazzard rootstocks, which are used only in limited numbers in New York, is probably even higher than that found in mahaleb. Surprisingly, the actual virus incidence of nursery trees in both certified and uncertified plantings (Table 4) is actually lower than that found in the rootstocks on which these trees were propagated. Two reasons for this decrease in virus incidence are obvious, *viz.,* (a) healthy buds inserted into diseased understocks are less likely to unite

and grow than healthy buds inserted on healthy understocks (Table 1), and (b) the major proportion of trees infected with sour cherry yellows are removed by horticultural inspectors.

Little can be done to alleviate the problem of rootstock contamination in commercial nurseries until suitable quantities of virus-free rootstocks become available. The production of such stone fruit rootstocks in commercial quantities is a difficult problem. Virus-free seed trees of desirable horticultural type must be produced, established, and maintained in virus-free condition for long periods of time. The production of such virus-free seed source trees has been initiated in New York and in other states, and increasing amounts of virus-free seeds and seedlings should become available within the next 7 to 10 years. Ultimately, certified trees will necessarily be propagated only upon certified roostocks.

## Discussion

The different species of cultivated stone fruits are attacked by a damaging group of virus diseases which, if contracted early in the life of a tree, result in considerable losses in fruit production. Since stone fruit trees are perennial plants with a normal life expectancy in New York of 20 years or more, the cumulative effect of such losses is particularly serious. Many of these virus diseases may be contracted in the nursery as new trees are propagated and the diseases become established in new orchards planted with the young trees. Although the symptoms of a few of the virus diseases of stone fruits are so readily evident that the diseases can be controlled by roguing in the nursery and in the orchard, several of the important diseases affecting stone fruits are not characterized by the presence of consistent diagnostic symptoms. Trees infected with viruses of this group of diseases may be planted unwittingly and may remain undetected by the fruit grower as long as the orchard exists. Such infected trees will not yield normally in spite of adequate cultural treatment and adequate control of insects and fungus diseases.

In addition to the losses these virus diseases cause in the orchard, they cause serious losses to the nurseryman. Propagation with diseased buds results in poor stands of nursery trees and in lowered grades of the infected budlings.

Little, if any, spread of virus diseases occurs in the nursery after propagation. Trees propagated on healthy understocks from healthy budwood remain virus-free for the time that they remain in the nursery. Certain of the viruses of stone fruits, however, spread from tree to tree in the orchard. In most cases, the rate of spread is so slow

that comparatively few new infections occur during the life of the orchard if healthy trees are initially planted, particularly if they are planted in fairly large solid blocks. Although the most damaging disease economically, sour cherry yellows, may spread in the orchard at a considerable rate under certain conditions, the rate is low unless a considerable reservoir of inoculum is located close by. Necrotic ring spot virus spreads in the orchard with great rapidity, but its effects on the orchard tree are much less damaging than those resulting from infection with the other stone fruit viruses.

In addition to diseases of virus origin, many varieties of stone fruits are affected by abnormalities that are of probable genetic origin. Certain of these abnormalities, such as leaf-casting mottle of prune and crinkle of sweet cherry, are widespread in occurrence. They reduce the productivity of affected trees considerably. None of these abnormalities has been found to be transmissible so that there is no spread from affected to healthy trees either in the orchard or in the nursery. They are, however, perpetuated if budwood from affected trees is used for propagation. Symptoms of several of these abnormalities are not pronounced in young nonfruiting trees but become evident when the tree reaches fruiting age.

Control measures for the virus diseases and genetic abnormalities affecting the cultivated stone fruits must be initiated as the new trees are propagated in the nursery. Simple visual inspection for symptoms of virus diseases or other abnormalities does not insure the selection of healthy budwood, since symptoms of many diseases are absent or obscure at the time of budwood collection. Several important virus diseases are seed-transmitted, but symptoms are usually lacking in seedling rootstocks when they are propagated. Satisfactory control of the virus diseases can be obtained only by the use of budwood and seedling rootstocks that have originated from indexed virus-free sources. Indexed budwood sources are currently available to commercial nurserymen. Although indexed seedling rootstocks are not yet available in sufficient quantity to supply commercial needs, they will become available within a few years.

The current nursery improvement program will not prevent all introductions of diseased trees into orchard plantings nor will it prevent all spread of stone fruit viruses in the orchard after healthy nursery trees are planted. It can be relied upon, however, to reduce the number of trees infected in the nursery and to decrease the rate of virus spread in the orchard to tolerable levels. Intensive research on stone fruit viruses is continuing, and new information will be made available as rapidly as it is obtained.

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